

Application No. 09/885,731  
Amendment Dated July 21, 2003  
Reply to Office Action of March 21,2003

**AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Currently Amended) A method of creating a nucleic acid multiplex, said method comprising the steps of:

1) creating a mixture comprising water, a Watson-Crick duplex, a sufficient number of single-stranded mixed base sequence molecules to form ~~a-the multiplex that includes~~including the Watson-Crick duplex, and an accelerator agent that increases a rate or amount of multiplex formation, said multiplex being a triplex or quadruplex, ~~wherein said single stranded molecule or molecules are selected so that, if in a multiplex, they would each be related to all other strands of the multiplex by adherence to base pairing rules, said rules being either Watson-Crick base-pairing rules or homologous binding base-pairing rules;~~ and

2) incubating said mixture to allow the multiplex to form, each strand of said multiplex related to all other strands of the multiplex by adherence to Watson-Crick base-pairing rules or homologous binding base-pairing rules;

provided that, within the multiplex, the Watson-Crick duplex added in step (1) is heteropolymeric with a G-C content between 10% and 90% and a combined frequency therein of purine-pyrimidine dimers and pyrimidine-purine dimers exceeds 25%.

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2. (Original) A method of Claim 1 wherein the multiplex created is a triplex, in step (1) the sufficient number of single-stranded molecules is 1, and in step (2) the triplex is formed.

3. (Original) A method of Claim 1 wherein the duplex substantially retains its double-helical structure and the single-stranded molecule resides in a groove of that double-helical structure.

4. (Currently Amended) A method of Claim 3-1 wherein the single-stranded molecule is related to one strand of the duplex by Watson-Crick base-pairing rules and to the second strand of the duplex by homologous binding base-pairing rules.

5. (Original) A method of Claim 4 wherein the duplex substantially retains its double-helical structure and the single-stranded molecule resides in a groove of that double-helical structure.

6. (Canceled) A method of Claim 1 where, within the multiplex, the Watson-Crick duplex added in step (1) is heteropolymeric with a G-C content between 10% and 90%, and furthermore the combined frequencies therein of purine-pyrimidine dimers and pyrimidine-purine dimers exceeds 25%.

7. (Currently Amended) A method of Claim 1 wherein steps (1) and (2) are performed with at least one of the nucleic acid strands and/or the duplexes not in a cell.

8. (Currently Amended) A method of Claim 1 wherein step (2) is performed without the assistance of a protein.

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9. (Currently Amended) A method of Claim 1 wherein in step (1), the water is added so that it accounts, on a volume basis, for at least 50 percent of ~~the~~a final volume of the mixture.

10. (Currently Amended) A method of Claim 1 wherein in step (1), the water is added so that it accounts, on a volume basis, for at least 80 percent of ~~the~~a final volume of the mixture.

11. (Original) A method of Claim 1 wherein in step (1), the water is added so that it accounts, on a volume basis, for all of the liquid added to the mixture.

12. (Currently Amended) A method of Claim 1 wherein step (2) is performed at a temperature or temperatures above ~~the~~a freezing temperature of the ~~aqueous solution mixture~~ and at not more than 85°C.

13. (Currently Amended) A method of Claim 12 wherein step (2) is performed at ~~at the~~ temperature or temperatures is/are between 5 °C and to 30 °C.

14. (Currently Amended) A method of Claim 13 wherein step (2) is performed at ~~at the~~ temperature or temperatures is/are between 15 °C and to 25 °C.

15. (Original) A method of Claim 1 wherein in step (1), a cation is added as the accelerator agent.

16. (Original) A method of Claim 15 wherein said cation is Na<sup>+</sup> provided at a concentration of 50mM to 125mM.

17. (Original) A method of Claim 15 wherein said cation is selected from the group

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consisting of  $Mn^{+2}$  provided at a concentration of 10mM to 45mM,  $Mg^{+2}$  provided at a concentration of 10mM to 45mM, and  $Ni^{+2}$  provided at a concentration of 20mM.

18. (Original) A method of Claim 1 wherein in step (1) an intercalator is added as an accelerator agent.

19. (Original) A method of Claim 18 wherein the intercalator is a fluorescent intercalator.

20. (Original) A method of Claim 19 wherein the fluorescent intercalator is selected from the group consisting of YOYO-1, TOTO-1, YOYO-3, TOTO-3, POPO-1, BOBO-1, POPO-3, BOBO-3, LOLO-1, JOJO-1, cyanine dimers, YO-PRO-1, TO-PRO-1, YO-PRO-3, TO-PRO-3, TO-PRO-5, PO-PRO-1, BO-PRO-1, PO-PRO-3, BO-PRO-3, LO-PRO-1, JO-PRO-1, cyanine monomers, ethidium bromide, ethidium homodimer-1, ethidium homodimer-2, ethidium derivatives, acridine, acridine orange, acridine derivatives, ethidium-acridine heterodimer, ethidium monoazide, propidium iodide, SYTO dyes, SYBR Green 1, SYBR dyes, Pico Green, SYTOX dyes, and 7-aminoactinomycin D.

21. (Original) The method of Claim 1 wherein the accelerator agent is a non-intercalating fluorophore.

22. (Original) A method of Claim 21 wherein the non-intercalating fluorophore is selected from the group consisting of biotin, rhodamine, Alexa dyes, BODIPY dyes, biotin conjugates, thiol-reactive probes, fluorescein and derivatives including but not limited to the caged probes, Oregon Green, Rhodamine Green, QSY dyes.

23. (Currently Amended) A method of Claim 1 wherein in step (1) the accelerator agent

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is an intercalator that binds to at least one of the minor groove and/or the major groove of the Watson-Crick duplex.

24. (Original) The method of Claim 1 wherein in step (1) the accelerator agent at 25 °C is a liquid.

25. (Original) The method of Claim 24 wherein in step (1) the accelerator agent is an organic liquid soluble in water.

26. (Original) The method of Claim 1 wherein in step (1) an accelerator agent that is a condensation agent as regards the Watson-Crick duplex is added.

27. (Previously Amended) The method of Claim 1 wherein in step (1) an accelerator agent that is a decondensation agent as regards the Watson-Crick duplex is added.

Claim 28 (Withdrawn).

29. (Currently Amended) A method of Claim 1 wherein the multiplex created is a quadruplex, in step (1) the Watson-Crick duplex is a first Watson-Crick duplex, and in step (1) the sufficient number of single-stranded molecules is 2, those single-stranded molecules are in a second Watson-Crick duplex, and in step (2) the quadruplex is formed from said first and second duplexes.—~~Preferably step (1) is done with the two single stranded molecules already in the second Watson Crick duplex.~~

Claims 30-55 (Withdrawn).